PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Form PCT/IPEA/416
346376/D21447		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IB2004/002505	29.06.2004	21.07.2003

International Patent Classification (IPC) or national classification and IPC
C12N15/62, C12N9/78

Applicant
TRANSGENE S.A. et al.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 7 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07.02.2005	14.12.2005

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/002505

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-35	as originally filed
Sequence listings part of the description, Pages	
1-3	as originally filed
Claims, Numbers	
1-36	received on 21.11.2005 with letter of 16.11.2005
Drawings, Sheets	
1/1	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/002505

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 35-36

because:

☒ the said international application, or the said claims Nos. 35-36 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2004/002505

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-36
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-36
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-34
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 35-36 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

For the assessment of the claims 35-36 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following document:
D1: US-A-5 545 548 (LINSLEY PETER ET AL) 13 August 1996 (1996-08-13)
D2: WO 99/54481 A (ERBS PHILIPPE ; TRANSGENE (FR); JUND RICHARD (FR)) 28 October 1999 (1999-10-28)

The International Preliminary Examination Report has been based on an assumed valid priority for the present application. Should the priority of the present application not be valid, the E document cited in the Search Report would be relevant with respect to novelty and inventive step (Article 33(2) and 33(3) PCT).

2 The subject-matter of the application referring to a polypeptide possessing CDase activity but no uracil phosphoribosyl transferase (UPRtase) activity, wherein the polypeptide is derived from a native CDase by addition of an amino acid sequence derived from a polypeptide possessing an UPRTase activity, appears to be new and

inventive.

D2 is regarded as being the closest prior art to the subject-matter. D2 discloses a fusion protein of CDase and UPRTase with CDase activity and UPRTase activity (see D2, SEQ ID NO:2 and example 5).
The subject-matter of the application therefore differs from the fusion protein disclosed in D2 in that the claimed fusion protein does not have UPRTase activity.
The subject-matter of the application is therefore novel (Article 33(2) PCT).

The problem to be solved by the present invention may therefore be regarded as the provision of an alternative polypeptide for gene therapy.
The solution to this problem proposed in the present application is considered as involving an inventive step (Article 33(3) PCT), because nothing in D2 alone or in combination with any of the other documents suggests the use of a polypeptide as proposed in the application in gene therapy.

Re Item VIII

Certain observations on the international application

1 The expression "substantially" used in claims 4, 7 and 9 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT. The said article requires that the claims, but not the description, shall define the matter for which protection is sought.

2 The expression "polypeptide has no UPRTase **or** thymidine kinase activity" used in claim 1 lacks clarity, because it is not clear whether it means that "the polypeptide has no UPRTase activity **and** no thymidine kinase activity" or that "the polypeptide can have UPRTase activity if it does not have thymidine kinase activity".

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IAP15 Rec'd PCT/PTO 20 JAN 2006
IB0402505

CLAIMS

1. Polypeptide possessing a CDase activity, characterized in that it is derived from a native CDase by addition of an amino acid sequence, with the proviso that said polypeptide has no UPRtase or thymidine kinase activity, wherein the amino acid sequence, added to the native CDase, derives from a polypeptide possessing an UPRTase activity.

2. Polypeptide according to claim 1, wherein the amino acid sequence, added to the native CDase, is linked to the C terminal end of the native CDase.

3. Polypeptide according to one of claims 1 to 2, characterized in that said polypeptide possessing an UPRTase activity derives from a yeast UPRTase, in particular that encoded by the Saccharomyces cerevisiae FUR1 gene.

4. Polypeptide according to claim 3, characterized in that the amino acid sequence, added to the native CDase, derives from an amino acid sequence which is substantially that depicted in SEQ ID NO: 2 sequence identifier, starting at the Ser residue in position 2 and finishing at the Val residue in position 216.

5. Polypeptide according to claim 4, characterized in that the amino acid sequence, added to the native CDase, is as depicted in SEQ ID NO: 2 sequence identifier, starting at the Ser residue in position 2 and finishing at the Val residue in position 216.

6. Polypeptide according to one of claims 1 to 5, characterized in that said native CDase is a yeast CDase, in particular that encoded by the Saccharomyces cerevisiae FCY1 gene.

7. Polypeptide according to claim 6, characterized in that the native CDase comprises an amino acid sequence which is substantially as depicted in SEQ ID NO: 1 sequence identifier, starting at the Met residue in position 1 and finishing at the Glu residue in position 158.

8. Polypeptide according to claim 7, characterized in that the native CDase comprises an amino acid sequence as depicted in SEQ ID NO: 1 sequence identifier, starting at the Met residue in position 1 and finishing at the Glu residue in position 158.

9. Polypeptide according to claim 6, characterized in that it comprises an amino acid sequence which is substantially as depicted in SEQ ID NO: 1 sequence identifier, starting at the Met residue in position 1 and finishing at the Val residue in position 373.

10. Polypeptide according to claim 9, characterized in that it comprises an amino acid sequence as depicted in SEQ ID NO: 1 sequence identifier, starting at the Met residue in position 1 and finishing at the Val residue in position 373.

11. Polypeptide according to one of claims 1 to 10, characterized in that it exhibits a CDase activity which is higher than that of said native CDase.

12. Nucleotide sequence which encodes a polypeptide according to one of claims 1 to 11.

13. Recombinant vector which carries a nucleotide sequence according to claim 12, placed under the control of the elements which are required for expressing it in a host cell.

14. Recombinant vector according to claim 13, characterized in that said vector is selected from the group consisting of plasmid and viral vectors, where appropriate combined with one or more substances which improve(s) the transfectional efficacy and/or the stability of the vector.

15. Recombinant vector according to claim 14, wherein said substance which improve the transfectional efficacy and/or the stability of the vector is selected from the group comprising cationic lipids, cationic polymers, lysophospholipides and polypeptides.

16. Recombinant vector according to Claim 14, characterized in that said vector is a viral vector which is derived from a pox virus, from an adenovirus, from a retrovirus, from a herpes virus, from an alphavirus, from a foamyvirus or from an adenovirusassociated virus.

17. Recombinant Vector according to claim 16, characterized in that said vector derived from a Modified Vaccinia Ankara (MVA) virus.

18. Recombinant Vector according to claim 17, characterized in that the nucleotide sequence according

to claim 12 is inserted at a site of a naturally occurring deletion within the MVA genome selected from the group consisting in deletion I, II, III, IV, V and VI.

19. Recombinant vector according to claim 18, wherein the site of the naturally occurring deletion is deletion III.

20. Recombinant vector according to one of claims 13 to 19, characterized in that the elements which are required for the expression comprise a promoter.

21. Recombinant vector according to claim 20, characterized in that the promoter is the promoter of the thymidine kinase 7.5K gene.

22. Recombinant vector according to claim 16, characterized in that said vector is an adenoviral vector which lacks all or part of at least one region which is essential for replication and which is selected from the E1, E2, E4 and L1-L5 regions.

23. Recombinant vector according to Claim 22, characterized in that said vector is an adenoviral vector which additionally lacks all or part of the non-essential E3 region.

24. Recombinant vector according to claim 20, characterized in that said promoter is the cytomegalovirus (CMV) early promoter.

25. Recombinant vector according to one of claims 13 to 24, characterized in that it additionally comprises

one or more genes of interest which is/are selected from the genes encoding interleukins IL-2, IL-4, IL-7, IL-10 and IL-12, interferons, tumor necrosis factor (TNF), colony stimulating factors (CSF) and factors acting on angiogenesis.

26. Recombinant vector according to claim 25, characterized in that the gene of interest encodes a polypeptide which is selected from IL-2 and INFγ.

27. Process for preparing a viral particle, wherein:

(i) a recombinant vector according to one of claims 13 to 25 is introduced into a complementing cell which is able to complement said vector in trans so as to obtain a tranfected complementing cell,

(ii) said transfected complementing cell is cultured under conditions which are appropriate for enabling said viral particle to be produced, and

(iii) said viral particle is recovered from the cell culture.

28. Viral particle which comprises a recombinant vector according to one of claims 13 to 26 or was obtained in accordance with the process according to claim 27.

29. Host cell which comprises a nucleotide sequence according to claim 12 or a recombinant vector according to one of claims 13 to 26, or which is infected with a viral particle according to claim 28.

30. Composition which comprises a polypeptide according to one of claims 1 to 11, a nucleotide sequence according to claim 12, a recombinant vector according to one of claims 13 to 26, a viral particle according to claim 28 or a host cell according to claim 29, in combination with a pharmaceutically acceptable excipient.

31. Composition according to claim 29, characterized in that it comprises a polypeptide according to one of Claims 1 to 11 and a second polypeptide of interest, in particular a polypeptide selected from IL-2 and INFγ.

32. Composition according to claim 30, characterized in that it comprises a nucleotide sequence according to Claim 12 and a second nucleotide sequence of interest which encodes a polypeptide selected from IL-2 and INFγ.

33. Therapeutic or prophylactic use of a polypeptide according to one of claims 1 to 11, of a nucleotide sequence according to claim 12, of a recombinant vector according to one of claims 13 to 26, of a viral particle according to claim 28 or of a host cell according to Claim 28 for preparing a medicament which is intended for treating the human or animal body by gene therapy or by administering protein which has been produced by the recombinant route.

34. Therapeutic use according to claim 33 for preparing a medicament which is intended for treating cancers, tumors and diseases which result from unwanted cell proliferation.

35. Method for treating diseases by gene therapy, characterized in that a nucleotide sequence according to claim 12, a recombinant vector according to one of claims 13 to 26, a viral particle according to claim 28 or a host cell according to claim 29 is administered to an organism or a host cell which is in need of such a treatment.

36. Method according to claim 35, or therapeutic use according to claim 33 or 34, wherein pharmaceutically acceptable quantities of a prodrug, advantageously an analog of cytosine, in particular 5-FC, are administered to said host organism or cell.